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Transition metal catalysts for the bioorthogonal synthesis of bioactive agents

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Abstract

The incorporation of abiotic transition metal catalysis into the chemical biology space has significantly expanded the tool kit of bioorthogonal chemistries accessible for cell culture and *in vivo* applications. A rich variety of homogeneous and heterogeneous catalysts has shown functional compatibility with physiological conditions and biostability in complex environs, enabling their exploitation as extracellular or intracellular factories of bioactive agents. Current trends in the field are focusing on investigating new metals and sophisticated catalytic devices and toward more applied activities, such as the integration of subcellular, cell- and site-targeting capabilities or the exploration of novel biomedical applications. We present herein an overview of the latest advances in the field, highlighting the increasing role of transition metals for the controlled release of therapeutics.

Addresses

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Bioorthogonal chemistry, Transition metal catalysis, Homogeneous catalysts, Heterogeneous catalysts, Prodrug activation.

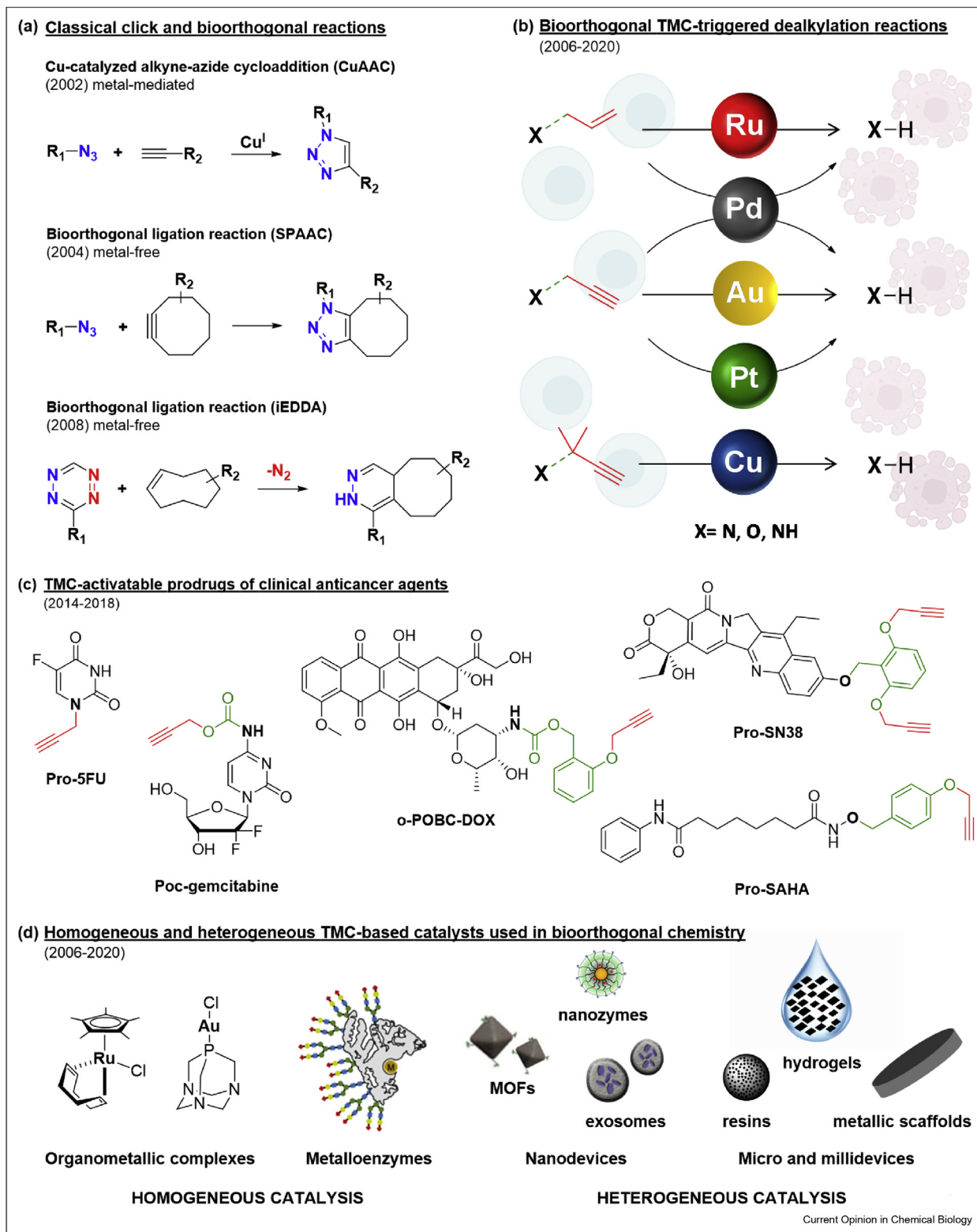
Introduction

Performing selective chemical modifications on biomolecules in their native conditions has challenged chemists for decades. Along with the constraints of adapting high-yielding reactions carried out in organic

solvents at high temperatures to aqueous media and lukewarmness, there is the essential requisite of procuring high chemoselectivity in the presence of a complex pool of functionalities. The field of bioorthogonal chemistry arose in response to this challenge by developing water-compatible reactions between reactant pairs with high reciprocal reactivity [1]. This has greatly expanded the research space for chemists and biologists, enabling to investigate and exploit previously unfeasible chemical transformations in conditions compatible with biomolecular structure, *in vitro*, in cells and in living organisms (Figure 1a). This synthetic paradigm continues to grow and evolve by the needs and interests of researchers from different areas [1,2].

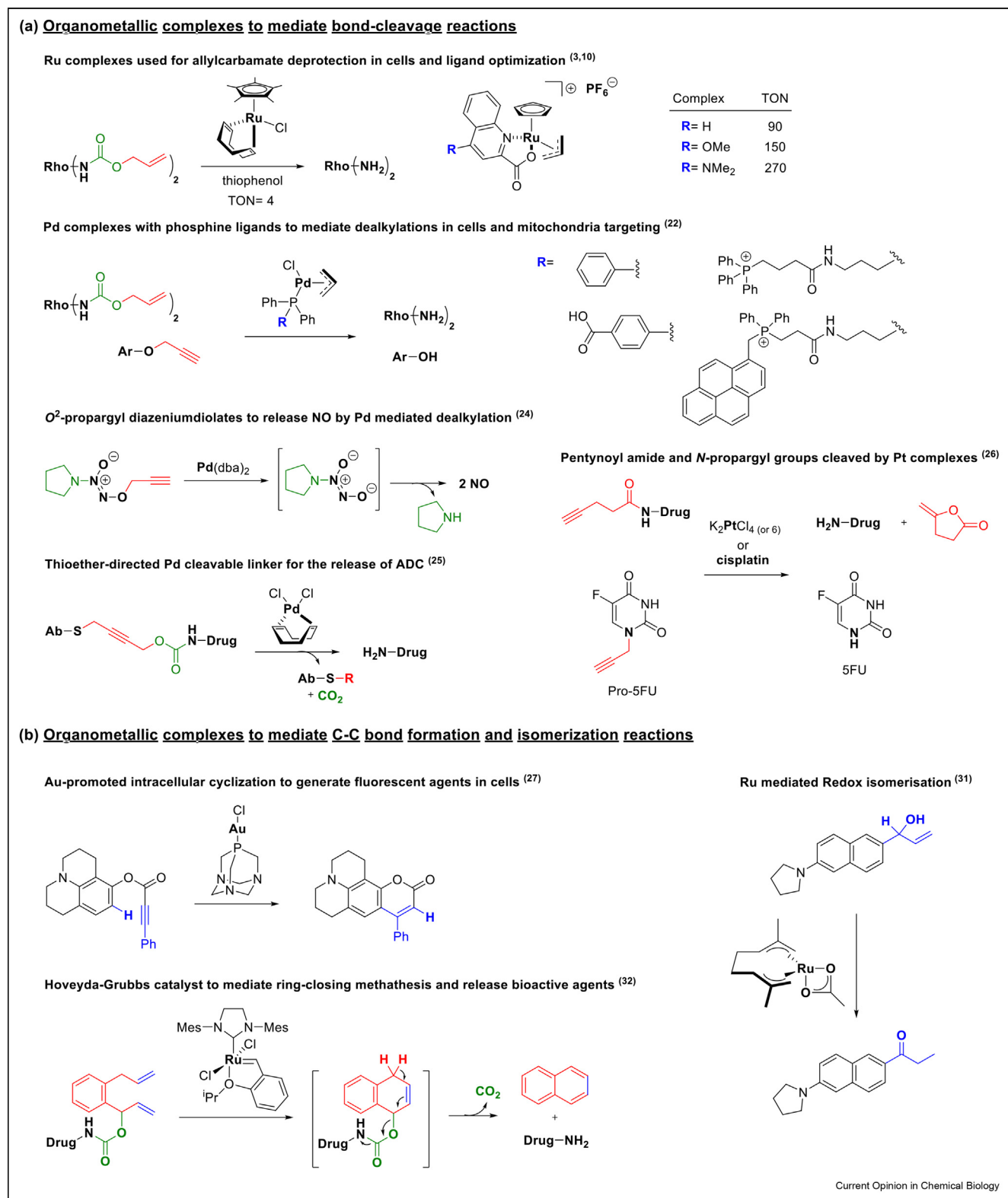
Among the new trends in the field, one exciting path taken by a number of laboratories have witnessed the integration of concepts and tools from the fields of transition metal catalysis, medicinal chemistry, and cell delivery under the bioorthogonal banner. Early incursions into the use of organometallic catalysts in living cells to perform specific xenobiotic transformations — rather than to directly elicit a pharmacological effect — explored the intracellular cleavage of allylcarbamate bonds using Ru [3] or Pd [4,5] catalysis (Figure 1b). Streu and Meggers [3] reported the intracellular release of Alloc-protected rhodamine 110 by an organometallic Ru(II) complex in HeLa cells in 2006. Later, Yusop et al. [4] and Unciti-Broceta et al. [5] proved this reaction could also be performed by Pd nanoparticles (Pd-NPs) captured at the surface of polystyrene beads (0.5 µm in diameter) that possessed the capacity to enter the cytoplasm of human cells [4,5]. Notably, these cell-penetrating Pd-functionalized nanodevices had the added capacity of mediating Suzuki–Miyaura cross-couplings, which was demonstrated inside cells with the synthesis of a mitochondria-targeted fluorophore. These early works sparked interest in the potential of using transition metal catalysts (TMCs) as bioorthogonal tools to unmask structures capable of mediating biological activity (Figure 1b and c). Thus, in 2014, independent works from Li et al. [6] and Weiss et al. [7,8] reported the capacity of Pd to mediate depropargylation reactions and investigated its use to control enzymatic activity (in cells) and to uncage clinical anticancer drugs (out of cells), respectively. In

Figure 1



Overview of bioorthogonal methods and tools. **(a)** Classical click and bioorthogonal reactions. **(b)** Bioorthogonal TMC-triggered dealkylation reactions. **(c)** Examples of TMC-activatable prodrugs of clinical anticancer agents. **(d)** Examples of homogeneous and heterogeneous TMC-based catalysts used in bioorthogonal chemistry.

Figure 2



Overview of organometallic complexes and reactions explored in bioorthogonal catalysis. **(a)** Organometallic complexes to mediate bond cleavage reactions. **(b)** Organometallic complexes to mediate C-C bond formation and isomerization reactions.

the same year, Sánchez et al. [9] and Volker et al. [10] reported that Ru-mediated deallylations could also trigger the uncaging of bioactive agents. Examples of TMC-activatable bioactive precursors include prodrugs of clinically used chemotherapeutics such as 5FU [7], gemcitabine [8], doxorubicin [11], SN38 (active metabolite of irinotecan [12]), or vorinostat [13] (Figure 1c). Many other TMCs capable of performing bioorthogonal reactions have emerged over the years [14–21], which have expanded the tool kit of metals and devices capable to release bioactive reagents or initiate enzymatic catalysis in living systems (Figure 1d).

Herein, we provide an up-to-date overview of the latest advances on the development and application of homogeneous and heterogeneous TMCs as bioorthogonal tools, with special emphasis on those devised to produce bioactive small molecules since 2018.

Organometallic complexes

A diversity of organometallic complexes has been investigated as bioorthogonal catalysts in recent years. The modular synthesis of this class of compounds facilitates the exploration of different oxidation states and multiple ligands to tune and optimize their physical, chemical, and biological properties, for example, water solubility, catalytic activity and productivity, cell penetrability, subcellular localization, and inherent toxicity. This is exemplified in the works of Streu and Meggers [3] and Volker et al. [10], which — after their early exploration of Ru-mediated deallylations in cells [3] — surveyed the use of quinolone-2-carboxylate ligands to optimize the catalytic properties of Ru(II) complexes under physiological conditions and in cells [10], resulting in improved catalysts' turnover numbers and eliminating the need of unnatural thiols as reaction additives (Figure 2a). Similarly, the exploration of phosphine-based ligands for the preparation of biocompatible Pd(II) complexes, reported by Martinez-Calvo et al. [22], led to the discovery of compounds capable of mediating intracellular dealkylation reactions and the incorporation of a ligand (bearing a pyrene ring) that induced selective complex accumulation in the mitochondria (Figure 2a). Using a submonomer solid-phase approach, Cherukaraveedu et al. [23] synthesized a library of *N*-heterocyclic carbene–Pd complexes that incorporated hydrophilic and lipophilic groups to investigate catalytic efficacy. The glycine-linked catalyst was the most robust catalyst of the series, being capable of activating Pro-5FU (a bioorthogonal prodrug of 5FU [7]) in cells and spheroids.

Conversely, other groups have focused on the development of new caging strategies and linkers to control the bioorthogonal release of bioactive agents by well-established organometallic compounds. In this

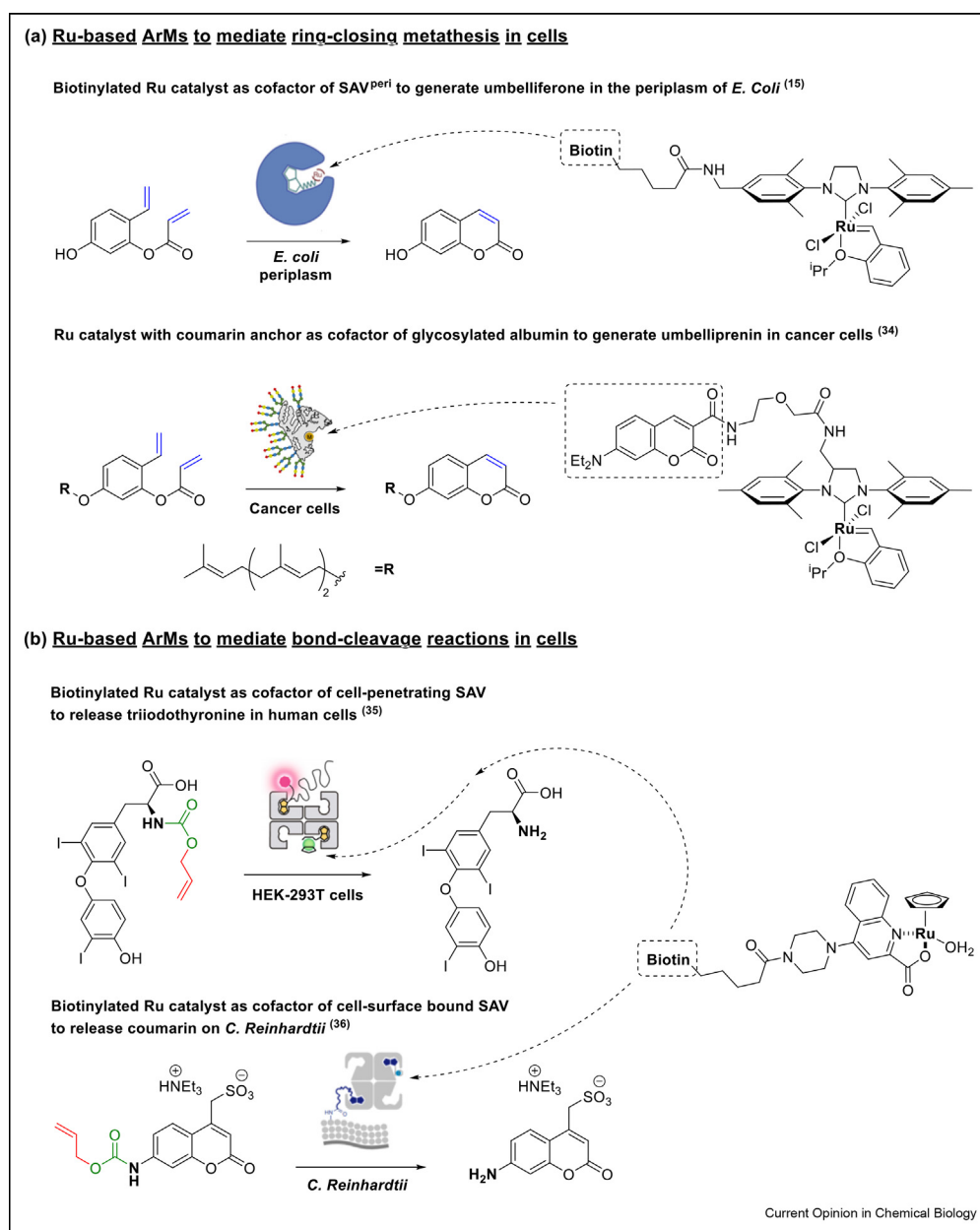
direction, Lv et al. [24] designed *O*²-propargyl diazeniumdiolates to control the release of NO molecules (natural metabolite that exerts antiproliferative activity at high concentration) by Pd-mediated dealkylation chemistry (Figure 2a). The variety of alkyne-bearing protecting groups that are labile to Pd catalysts was supplemented with the development of a thioether-directed cleavable linker by Stenton et al. [25] (Figure 2a). This linker was used to construct an antibody–drug conjugate (ADC) capable of releasing its cytotoxic cargo by Pd chemistry, specifically an anti-HER2 nanobody–doxorubicin conjugate that is cleaved upon reaction with Pd(COD)Cl₂. More recently, the same group has reported that Pt complexes (II and IV) can also cleave alkyne-bearing masking groups (pentynoyl amide and *N*-propargyl groups) in living systems and be used to release caged drugs and cleave ADCs (Figure 2a) [26]. Of note, the chemotherapeutic drug cisplatin (a Pt (II) complex) was capable of activating Pro-5FU [7], resulting in tumor shrinkage in a xenograft model in zebrafish only when both drug and prodrug were added together [26].

Along with bond cleavage reactions, Au- and Ru-based organometallic complexes have been used to mediate different bioorthogonal transformations in cells, such as bond formation and isomerization reactions (Figure 2b). Vidal et al. [27] showed the capacity of an Au(I) complex (featuring an 1,3,5-triaza-7-phosphaadamantane ligand) to promote the hydroarylation of a procoumarin substrate in cells to generate a highly fluorescent fluorophore (Figure 2b). In this study, the use of 1,3,5-triaza-7-phosphaadamantane and other water-soluble ligands was investigated, with the former showing optimal reactivity in cells and lower toxicity than the others, showcasing the importance of the ligand to generate a truly bioorthogonal organometallic complex. Researchers have also shown that cyclometalated Au(III) complexes can catalyze cysteine arylation reactions in peptides under physiological conditions [28,29], a process that may play a role in the cytotoxic effect of certain organogold compounds. Nonetheless, as reported by Wenzel et al. [29], the selection of the ligand is key to drive this cross-coupling reaction toward the modification of certain zinc finger domains. The authors found that selected Au(III) complexes featuring bidentate C[^]N-donor ligands are optimal to form adducts with the Cys residues of zinc finger peptides (Cys₂His₂ type) and, subsequently, create a C–S bond between the target peptide and the ligand by reductive elimination. The possibility to direct this cross-coupling process to specific sequences of biologically relevant proteins makes this reaction a promising addition to the bioorthogonal toolbox [30]. Vidal et al. [31] recently demonstrated that Ru(IV) complexes can catalyze redox-neutral isomerization reactions in living cells, enabling the intracellular transformation of

nonbiological allylic alcohols into α,β -unsaturated ketones (Figure 2b). This strategy was also used to generate α,β -unsaturated ketones with strong Michael acceptor character to deplete the cells from protective nucleophilic thiols such as glutathione, which represents a new strategy to induce a biological effect through a bioorthogonal process. Using the same metal, Sabatino et al. [32] reported in the same year a completely different approach to modulate cell biology in a bioorthogonal manner. The authors designed a masking group that is cleaved in two sequential steps

triggered by a single Ru-mediated reaction: ring-closing metathesis followed by drug release driven by the generation of an aromatic ring (Figure 2b). The so-called ‘close-to-release’ strategy performed best using the water-soluble 2nd generation Hoveyda–Grubbs catalyst and is applicable to protect various functionalities [32]. Of note, caged umbelliferone was successfully released using an artificial metathase (protein functionalized with Ru complex previously developed by the same group [15]) in *E. coli*. This distinct class of catalysts is described in detail in the following section.

Figure 3



Overview of the modular design of ArMs used in cells and their reactivities. **(a)** Ru-based ArMs to mediate ring-closing metathesis in cells. **(b)** Ru-based ArMs to mediate bond-cleavage reactions in cells.

Artificial metalloenzymes

Metalloproteins developed in the laboratory — so-called artificial metalloenzymes (ArMs) — are becoming essential biotechnology tools in research and industry [33]. The opportunity of upgrading nonbiological TMCs with enzyme-like substrate specificity and high biocompatibility has recently inspired chemists to explore ArMs for performing bioorthogonal catalytic reactions. Although several TMCs have been investigated (e.g. Au, Rh, Ir [33]), Ru has been the main metal explored to develop ArMs with the capacity to manufacture bioactive agents in cell culture [34–36]. The earliest use of ArMs to mediate a bioorthogonal reaction in cells was reported by Jeschek et al. [15] in 2016. In this work, a derivative of the 2nd generation Hoveyda–Grubbs catalyst was conjugated to biotin, which acted as an anchor to incorporate this TMC into streptavidin (SAV) and thereby create an ArM with the capacity to catalyze ring-closing metathesis (Figure 3a). Design of an *E. coli* strain expressing SAV fused with the OmpA signal peptide (SAV^{peri}) enabled protein accumulation in the cell periplasm. The ArM was formed *in situ* upon addition of the Ru–biotin complex, endowing the cells with metathesis activity as demonstrated by the synthesis of umbelliferone. While the reaction yield was modest, the authors demonstrated its improvement through directed evolution, a method that also allowed optimization for different substrates [15].

The assembly of ArMs to promote Ru-catalyzed metathesis was also investigated by Eda et al. [34] (Figure 3a). The goal of this work was to create an ArM that was unaffected by intracellular thiols. To do so, they exploited the high binding affinity of the deep hydrophobic pockets of albumin for aminocoumarin to anchor a Ru catalyst into glycosylated human serum albumin. This glycosylated ArM, equipped with Ru(II) and modified with *N*-glycan targeting moieties, mediated ring-closing metathesis of a range of diene compounds, including a precursor of umbelliprenin (natural product with anticancer properties). In this approach, cancer cells are preferentially targeted by the glycans of the ArMs, facilitating the selective synthesis of anticancer agents in malignant cells [34].

A biotinylated derivative of the catalyst developed by Volker et al. [10] has also been used to build artificial deallylases (Figure 3b) [35,36]. Okamoto et al. [35] functionalized SAV with a module that promoted cell penetration (poly-arginine functionality) and a Ru(II) catalyst to catalyze the intracellular uncaging of the thyroid hormone triiodothyronine. This bioactive molecule was generated in HEK-293T cells transfected with a hormone-responsive gene switch, thereby inducing the expression of a secreted NanoLuc luciferase [35]. In a different strategy, Szponarski et al. [36] used a dimeric SAV to bind the protein onto the surface

of *C. reinhardtii* (via membrane protein conjugation with a biotinylated reagent) followed by anchoring a Ru(II)–biotin complex to facilitate the site-selective extracellular release of aminocoumarin (Figure 3b).

Nanodevices

As discussed in the introduction, the first example of a miniaturized device able to perform artificial chemistries in cells was reported by Yusop et al. [4] in 2011, who used polystyrene-based supports of approx. 0.5 μm in diameter functionalised with Pd-NPs to mediate deallylation and Suzuki reactions in the cell cytoplasm. Subsequent studies by other groups were addressed to further the understanding of how to control the activity and localization of TMCs through nanodevice design. In 2015, Tonga et al. [14] developed a new design based on the use of Au-NP cores and self-assembling monolayers to facilitate the incorporation of hydrophobic Ru and Pd complexes within the lipophilic monolayer of the devices. By sterically blocking their surface with macrocyclic cucurbiturils bound onto supramolecular anchors tethered to the so-called nanozymes, they achieved bioorthogonal regulation of TMC activity: addition of a guest molecule (1-adamantylamine) resulted in the displacement of the anchoring groups from the hosting pocket of the cucurbiturils, thus freeing the nanozyme surface and enabling the entry of prodyes and prodrugs to undergo dealkylation reactions. Harnessing the reversible photoisomerization of azobenzene switches and the capacity of β -cyclodextrin to host *trans*-azobenzene but not *cis*-azobenzene, Wang et al. [37] developed a light-controlled bioorthogonal catalytic system based on mesoporous silica nanoparticles functionalized with Pd-NPs, complexed with azobenzene groups and superficially caged with β -cyclodextrins. Alike the natural allosteric regulation of an enzyme, catalytic activity was controlled in a reversible manner by light: activation under UV light and deactivation under visible light. Its applicability in cells was shown with the synthesis of two fluorescent probes — by dealkylation and Suzuki reactions — and the conversion of Pro-5FU into cytotoxic 5FU.

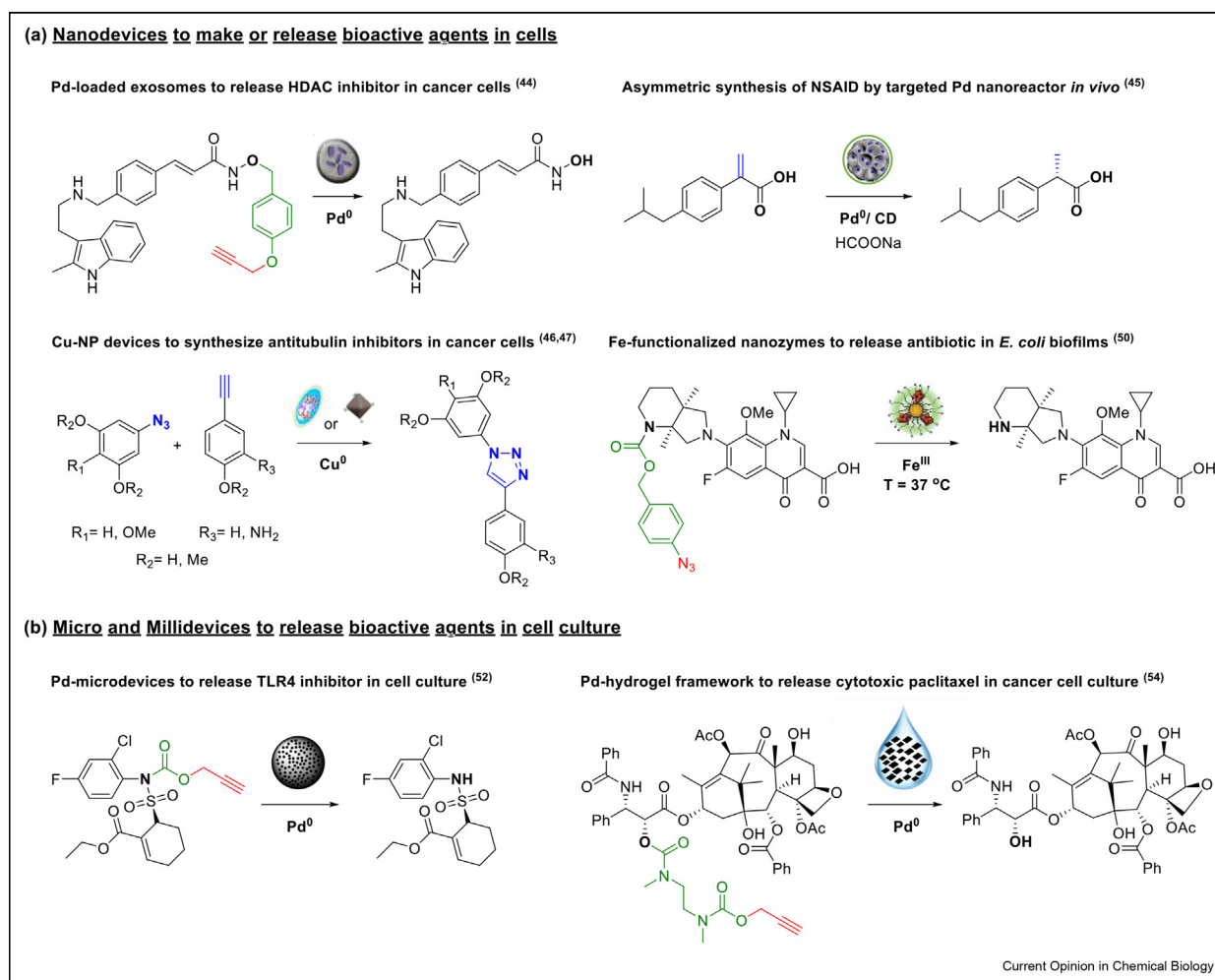
Following on from his original work on nanozymes, the Rotello lab has continued exploring alternative chemical motifs at the surface of this class of nanodevices to dictate intracellular or extracellular nanozyme localization [38] and to determine the effect of hard and soft protein coronas on the catalytic activity of the nanozymes [39]. This last study showed that hard ‘irreversible’ coronas sterically block the TMC activity of the nanozymes, while being restored after endosomal cell entry and proteolytic degradation, thereby offering a method to confine the bioorthogonal manufacture of xenobiotics to the intracellular space. Other researchers such as Destito et al. [40] and Martinez et al. [41] have focused their efforts on improving the

productivity of bioorthogonal Pd catalysis through the design of different kinds of nanodevices. Destito et al. [40] developed hollow silica microspheres (0.5 μm in diameter) as supports to protect Pd-NPs from passivation and poisoning by biomolecules. These devices were highly efficient in performing depropargylation reactions in biorelevant aqueous media and cells. More recently, Martinez et al. [41] reported a water compatible core-shell Pd/metal-organic framework nanocomposite that promoted depropargylations to activate different prodrugs in cells and 3D spheroids. Cells loaded with these nanodevices were able to process sequential batches of reacting probes (up to 4 cycles), first example of reusable ‘catalytic cells’.

One of the key advantages of using heterogeneous or encapsulated TMCs into nanoscale carriers is the potential to achieve intratumoral accumulation *in vivo* through the enhanced permeability and retention effect.

Miller et al. [17] demonstrated this using poly(lactic-co-glycolic acid)-polyethyleneglycol (PLGA-PEG) polymeric micelles to encapsulate Pd-Cl₂ (TFP)₂ and deliver the catalyst into tumor xenografts. Separate administration of an Alloc-protected prodrug of doxorubicin [10] (also encapsulated in PLGA-PEG polymeric micelles) achieved site-selective delivery of both formulations, which upon reaction elicited anticancer activity in a fibrosarcoma model in mice. The versatility of this approach was expanded *in vivo* with the delivery of an encapsulated prodrug of an extremely potent toxin such as monomethyl auristatin E [42]. Using a different targeting concept, Hoop et al. [43] also reported a strategy to achieve site-selective delivery of Pd catalysts in culture and *in vivo* by creating nanowires composed of magnetic Fe and catalytic Pd. Magnetic fields were used to localize the devices to predefined areas of a cancer cell culture and selectively kill the targeted cell population by activation of Pro-5FU [7] into 5FU. In a proof-of-concept assay, the authors showed the *in vivo* potential

Figure 4



Nanodevices, microdevices, and millidevices used to generate bioactive compounds in cells and organisms. **(a)** Nanodevices to make or release bioactive agents in cells. **(b)** Microdevices and millidevices to make or release.

of this approach to induce Pro-5FU activation and tumor shrinkage in a xenograft model directly inoculated with the magnetic nanocatalysts.

Increasing the delivery efficiency of therapeutics to disease sites, thereby preventing damage to healthy tissues, is one of the main goals of the nanomedicine field. Beyond the aforementioned studies, which addressed site-selective targeting by physical methods, a recent study by Sancho-Alberto et al. [44] has shown the potential of using intercellular trafficking pathways to carry catalytic nanoreactors to specific cancer cells. The authors developed a bioinorganic hybrid nanoplat-form for targeted bioorthogonal chemistry by integrating the targeting capabilities of cancer-derived exosomes and the catalytic properties of Pd nanosheets [44]. These Pd-functionalized vesicles showed preferential tropism for their parent cells and mediated the intra-cellular manufacture of the anticancer drug panobino-stat in their progenitor cells but not in other cell lines (Figure 4a). Du et al. [45] proposed a different approach to achieve site-selective delivery of TMCs. The authors supported Pd-NPs on mesoporous silica nanoparticles and cloaked them with neutrophil membranes to be trafficked to sites of inflammation. Notably, the catalyst surface was modified with cinchona alkaloids to promote asymmetric transfer hydrogenation in the presence of hydrogen donors (sodium formate). Enantioselective synthesis of *S*-ibuprofen (active enantiomer) from a nonchiral precursor was carried out *in vivo*, alleviating the inflammation of injured mouse paw.

Alternative transition metals such as Cu, Au, and Fe have recently been used in the development of bioorthogonal catalytic nanodevices. Lui et al. [20] used single-chain polymeric nanoparticles modified with metal-complexing ligands as polymeric scaffolds to bind Pd(II) or Cu(I) ions. Of note, they showed that dime-thylpropargyl groups were rapidly cleaved by Cu(I)-based single-chain polymeric nanoparticles in cells, chemistry that was later modified by Wang et al. [21] to catalyze the bioorthogonal cleavage of ADCs with Cu complexes. Huang et al. [46] encapsulated Cu-NPs in cross-linked lipolic acid nanoparticles of various mor-phologies to perform CuAAC reactions in cells. Inter-estingly, devices with a prolate spheroid shape were taken up faster by cells and catalyzed the synthesis of a triazole-containing anticancer agent (drug synthesis strategy first reported by Clavadetscher et al. [18], Figure 4a). Wang et al. [47] built a heterogeneous Cu catalyst on a metal–organic framework decorated with lipophilic cations to perform CuAAC reactions in the mitochondria of cancer cells, where they mediated the synthesis of an polyphenolic analog of the same triazole-containing bioactive agent (Figure 4a).

Introducing additional control into bioorthogonal nano-reactors, Kumar et al. [48] reported that the entrapment

of plasmonic Au nanospheroids in aminated silica nanoparticles can efficiently harness NIR light to accelerate catalysis and allow remote control of catalytic activity in living cells. Through an alternative photo-catalytic strategy, Mazzei et al. [49] proposed the use of Au-NPs functionalized with alkanethiols bearing 1,4,7-triazacyclononane headgroups (positively charged under physiological conditions) to encapsulate nega-tively charged flavins. Under UV irradiation in the presence of 2-(*N*-morpholino)ethanesulfonic acid (electron donor), these nanodevices were capable of reducing a Pt (IV) prodrug into the Pt (II) anticancer drug cisplatin, although the compatibility of these photocatalytic nanozymes in cells is yet to be demon-strated. Of note, in this approach, the role of the metals are inverted: Au-NPs act as scaffolds to build the nanozymes that entrap the flavins, which are the photocatalysts, and the Pt (IV) species are the reaction substrates. Using thermal control instead, Cao-Milan et al. [50] developed a thermoresponsive nanozyme by incorporating supramolecular assemblies of Fe(III)-porphyrins (FeTPP). At low temperature, the FeTPP complexes aggregate and become catalytically inactive, while the FeTPP disassembles upon heating throughout the lipidic monolayer, enabling their catalytic properties in a reversible manner. Its applicability was demon-strated by reducing a prodrug to release the antibiotic moxifloxacin and effectively killing *E. coli* bacterial bio-films (Figure 4a).

Extracellular microdevices and millidevices

In 2014, Weiss et al. [7,8] reported the use of extra-cellular microdevices (TentaGel resins) to entrap Pd-NPs and mediate bioorthogonal drug activation in cell culture. The authors showed that the Pd-mediated cleavage of propargyl groups took place faster than that of allyl groups and proved their *in vivo* functional compatibility in zebrafish. This solid-supported approach was later expanded to host other metal nano-particles such as Cu (Clavadetscher et al. [18]) or Au (Perez-Lopez et al. [19]) to make anticancer agents by CuAAC and depropargylation reactions, respectively, or to mediate extracellular Pd-promoted Suzuki reactions to assemble a multikinase inhibitor [51]. More recently, Bray et al. [11] studied the importance of the size of the microspheres and found that 30 μm in diameter was optimal for Pd loading capacity and catalytic efficacy. This size was also suitable for intratumoral implantation into an orthotopic prostate cancer in mice. Notably, the authors showed that — because of the echogenic nature of the metal — the insertion of the implants could be monitored by ultrasound-guided imaging (a standard clinical procedure). By tumor resection and monitoring *ex vivo*, the authors proved the activation of a fluorescent probe and a prodrug of doxorubicin (oPOBC-Dox, Figure 1c) and that the devices remained unaltered and catalytically active after 21 days in the tumor. Adam

et al. [12] later showed that these devices were capable to activate simultaneously two prodrugs in cancer cell culture, Pro-SN38 and Pro-5FU (Figure 1c), cytotoxic agents clinically used in combination therapy. Toward a different application, Plunk et al. [52] proposed the use of bioorthogonal Pd chemistry to induce local suppression of the innate immunological response after organ transplants. Although still in an early stage, they reported the use of Pd microspheres to activate a bioorthogonal prodrug of resatorvid (Figure 4b), a toll-like receptor 4 inhibitor in clinical development [52].

The use of millimeter-sized scaffold materials to host Pd catalysts has also been explored. Torres-Sánchez et al. [53] manufactured implants by depositing Pd onto titanium scaffolds using different techniques. These devices showed high biocompatibility in cancer cell culture and the capacity to activate a prodrug of the anticancer drug vorinostat (Pro-SAHA [13], Figure 1c). Toward the development of implantable catalytic systems that are inherently biodegradable, Perez-López et al. [54] reported the use of natural hydrogels (agarose and alginate) to entrap ultrathin Pd nanosheets in solid polymeric frameworks that allow the internal diffusion of small molecules (Figure 4b). This strategy provides a catalytic system to temporarily promote local bioorthogonal reactions, which would be ideal for short-term applications (e.g. neoadjuvant chemotherapy). Of note, the authors designed a Pd-activatable prodrug of the anticancer drug paclitaxel (Figure 4b) using a protecting group tailored made for aliphatic OH.

Conclusions

The use of metal catalysts in biomedical research has continuously grown during the last decade. Alongside strategies that exploit TMCs to promote the catalytic transformation of cell substrates and achieve a direct biological effect, so-called catalytic metallodrugs [55,56], applications have greatly expanded toward the opposite direction, that is, the use of abiotic metals as bioorthogonal catalysts to mediate new-to-life reactions in a safe manner. The toolbox of TMCs that shows innocuity and functional compatibility with living cells and organisms is rapidly increasing, allowing researchers to explore multiple chemical processes and biological applications, including the bioorthogonal synthesis of a variety of pharmacological agents. This rapidly evolving research topic represents nowadays a key driver in chemical biology, as seen by the continued appearance of innovative works, even during the preparation of this review [57–59].

One of the key aspects of this research is the use of homogeneous versus heterogeneous catalysts, a choice that is made based on the goals of the study. The first ones have the advantage of increasing catalyst productivity and can be incorporated into metalloproteins

to improve their biocompatibility and substrate specificity. On the other hand, heterogeneous TMC devices can modulate best the fate of the catalyst *in vivo* and act either as a targeted or implantable bioorthogonal reactor for therapeutic applications. While the latest advances in this direction have shown great promise to progress the use of bioorthogonal TMCs into a *bona fide* medical alternative, future developments will need to focus further on fine-tuning target selectivity and improving long-lasting catalytic activity *in vivo*. Shielding the TMCs from direct contact with intercellular/intracellular environments will likely be essential to prolong the catalyst's lifespan and thereby achieve bioactive levels of drug release at the disease site. Importantly, the development of new methods and TMC-labile sensors will be vital to monitor catalyst activity *in vivo*, as a proxy to measure its productivity and assist adequate matching with (pro)drugs that display the right level of activity. Combined administration of catalytic devices that display high *in vivo* productivity and a precursor of an ultrapotent cytotoxic agent could generate supratherapeutic drug levels that could distribute to healthy organs and cause harm. On the contrary, low-productive catalysts should not be combined with prodrugs of chemotherapeutics of low-to-moderate activity, as this strategy will likely generate local levels of drug below the therapeutic threshold. All these aspects will need to be confronted on the pathway from preclinical research to clinical development. The success to translate the use of TMCs as bioorthogonal medical devices will depend on our capacity to develop strategies and methods that address them. The challenge is set.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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